(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 October 2003 (23.10.2003)

PCT

(10) International Publication Number
WO 03/086391 A1

(51) International Patent Classification[7]: A61K 31/42, 31/275, A61P 37/06

(21) International Application Number: PCT/JP03/04722

(22) International Filing Date: 14 April 2003 (14.04.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/372419 16 April 2002 (16.04.2002) US

(71) Applicant *(for all designated States except US)*: **FUJISAWA PHARMACEUTICAL CO., LTD.** [JP/JP]; 4-7, Doshomachi 3-chome, Chuo-ku, Osaka-Shi, Osaka 541-8514 (JP).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KOBAYASHI, Masakazu** [JP/US]; 1800 Dewes Court #310, Glenview, IL 60025 (US). **JIANG, Hongsi** [CN/US]; 2630 Old Glenview Road, Wilmette, IL 60091 (US). **PAN, Fan** [CN/US]; 211 Valley View Dr., Wilmette, IL 60091 (US). **ERICKSON, Laurie** [US/US]; 2506 W. Morse Avenue, Chicago, IL 60645 (US). **EBBS, Aaron** [US/US]; 113 Caraway Rd,, Apt. 2C, Reisterstown, MD 21136 (US). **WYNN, Carmen** [US/US]; 1918 W. Morse Avenue, #1-N, Chicago, IL 60626 (US).

(74) Agent: **TABUSHI, Eiji**; Fujisawa Pharmaceutical Co., Ltd., Osaka Factory, 1-6, Kashima 2-chome, Yodogawa-ku, Osaka-shi, Osaka 532-8514 (JP).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

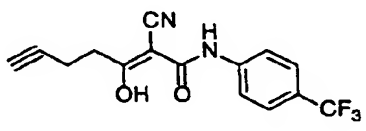
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

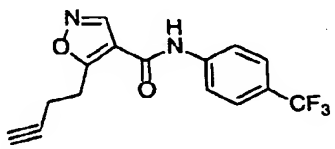
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/086391 A1

(54) Title: NEW USE



(I)



(II)

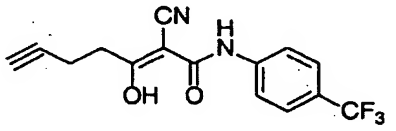
(57) Abstract: This invention relates to a new use of a compound of the following formula (I) or (II) for the manufacture of a medicament for preventing and/or treating chronic rejection in a transplanted organ or tissue.

DESCRIPTION

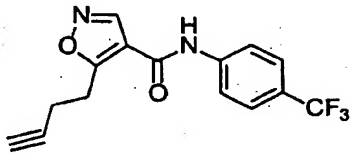
MEDICAMENT FOR PREVENTING AND/OR TREATING CHRONIC REJECTION

Technical Field

This invention relates to a new use of a compound of the following formula (I) or (II) for the manufacture of a medicament for preventing and/or treating chronic rejection in a transplanted organ or tissue.



(I)



(II)

Background Art

Organ transplants of liver, kidney, lung and heart are now regularly performed as treatment for endstage organ disease. Transplant outcome has progressively improved with the development of refinements in tissue typing, surgical techniques, and more effective immunosuppressive treatments. However, because of problems with chronic rejection, organ transplantation is not yet a clinically viable solution to irreversible organ disease.

Chronic rejection, which manifests as progressive and irreversible graft dysfunction, is one of the leading causes of late organ transplant loss in clinical transplantation.

The typical chronic rejection with the prognosis is an arteriosclerosis-like alteration, such as transplant

vasculopathy, graft vessel disease, graft arteriosclerosis, transplant coronary disease, angiostenosis, interstitial fibrosis, etc. This vascular lesion is characterized by migration and proliferation of smooth muscle cells, namely, this leads to intimal proliferation and thickening, smooth muscle cell hypertrophy repair, and finally to gradual luminal obliteration (vascular remodelling). Especially, in the case of kidney, chronic rejection may be called chronic allograft nephropathy.

Chronic rejection appears to be inexorable and uncontrollable because there is no known effective treatment or prevention modality. Thus, there continues to exist a need for a remedy effective in preventing and/or treating chronic allograft rejection in clinical organ transplantation.

Concerning the compound (I) or (II) used in the present invention, it is known that the compound (I) or (II) is useful for the treatment of rheumatoid arthritis, chronic inflammatory diseases of immune or non-immune origin, and cancer in USP 5,308,865. While chronic inflammatory disease is disclosed in this patent, it is different from chronic rejection in a transplanted organ characterized by vascular lesion, so chronic rejection in a transplanted organ is not disclosed.

It is known that leflunomide and related compounds reduce overproliferation of smooth muscle cell following vascular injury, accordingly these compounds are useful for prevention and treatment of angiostenosis and arteriosclerosis following vascular injury in EP 0665013. However, the compound (I) or (II)

of the present invention is not disclosed in the patent application. Additionally, chronic rejection in the present invention is discovered in whole vessel of transplanted organ as a result of host immune and non-immune responses, while the disease described in the patent application appears in injured part for damage restoration. So, these diseases are completely different on embryology in each other.

It is known that general leflunomide compounds have activities to control or reverse chronic rejection in a transplanted organ in USP 5,624,946 and USP 5,688,824. However, the compound (I) or (II) of the present invention is not disclosed in these patents.

Accordingly, it is not known at all that the compound (I) or (II) has activity to prevent and/or treat chronic rejection in a transplanted organ or tissue.

Disclosure of Invention

The inventors of this invention have found that the compound (I) or (II) is effective for preventing and/or treating chronic rejection in a transplanted organ or tissue in a mammalian recipient.

Accordingly, this invention provides a new method for preventing and/or treating chronic rejection in a transplanted organ or tissue, which comprises administering a therapeutically effective amount of the compound (I) or (II) to a mammalian recipient in need thereof.

Further, this invention provides a new use of the compound (I) or (II) for the manufacture of a medicament for preventing and/or treating chronic rejection in a transplanted organ or tissue.

Still further, this invention provides a new pharmaceutical composition for preventing and/or treating chronic rejection in a transplanted organ or tissue, which comprises a therapeutically effective amount of the compound (I) or (II) in admixture with a pharmaceutically acceptable carrier or excipient.

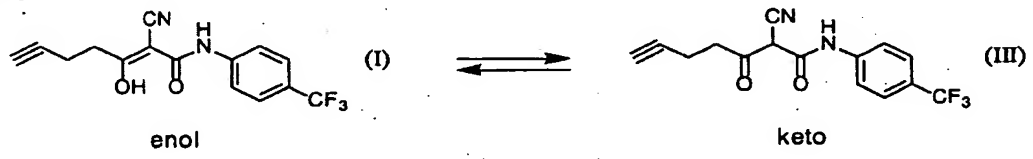
A remedy capable of preventing chronic rejection is a remedy that prevents the occurrence of functional or histological signs of chronic rejection, when initiated before chronic rejection has commenced either by long term or short term administration. Therefore, preventing chronic rejection used in the present invention means protection or maintenance of transplanted organ or tissue for a long term.

The term "treatment" used in this invention means both treatments that comprise "controlling" and "reversing" the disease. And a treatment capable of controlling chronic rejection is a treatment that slows the progression of the disease process, when initiated after functional or histological signs of chronic rejection, respectively, are observed. Further, a treatment capable of reversing chronic rejection is a treatment that, when initiated after functional or histological signs of chronic rejection (respectively) have appeared, reverses the disease process and returns functional and histological findings closer

to normal.

With respect to the compound (I), i.e. (2Z)-2-cyano-3-hydroxy-N-[4-(trifluoromethyl)phenyl]-2-hepten-6-ynamide, or the compound (II), i.e. 5-(3-butynyl)-N-[4-(trifluoromethyl)phenyl]-4-isoxazolecarboxamide, of the present invention, it can be produced according to the description in USP 5,308,865, Example 14 or a similar manner thereof, and it is to be understood that there may be a conformer and a stereoisomer, and such conformer and isomer are also included within the scope of this invention, and the compound (I) can be in another tautomer form. For example, the compound (I) can be either in its enol (I) or keto form (III), i.e. 2-cyano-3-oxo-N-[4-(trifluoromethyl)phenyl]-6-heptynamide, as shown in the following Scheme, and such a tautomer form is also included within the scope of this invention.

Scheme



The compound (I) or (II) can be in a solvate, which is included within the scope of the present invention. The solvate preferably includes a hydrate and an ethanolate.

The compound (I) or (II) in the present invention can be used in the form of a pharmaceutical preparation, for example, in solid, semisolid or liquid form, which contains the compound (I) or (II) as an active ingredient, in admixture with an organic or inorganic carrier or excipient suitable for oral, parenteral

such as intravenous, intramascular, subcutaneous or intraarticular, external such as topical, enteral, intrarectal, transvaginal, inhalant, ophthalmic, nasal or hypoglossal administration. The active ingredient may be compounded, for example, with the usual non-toxic, pharmaceutically acceptable, carriers for tablets, pellets, capsules, eye drops, suppositories, solutions (saline, for example), emulsion, suspensions (olive oil, for example), ointment, aerosol sprays, cream, skin plasters, patches and any other form suitable for use. The carriers which can be used are water, glucose, lactose, gum acacia, gelatin, mannitol, starch paste, magnesium trisilicate, corn starch, keratin, colloidal silica, potato starch, urea and other carriers suitable for use in manufacturing preparations, in solid, semisolid, or liquid form, and in addition auxiliary, stabilizing, thickening and coloring agents and perfumes may be used. The active object compound is included in the pharmaceutical composition in an effective amount sufficient to prevent and/or treat chronic rejection in a transplanted organ or tissue.

Mammals which may be treated in the present invention include livestock mammals such as cows, houses, etc., domestic animals such as dogs, cats, rats, etc. and humans, preferably humans.

Organs or tissues may be transplanted from a donor to a recipient of same individual (autograft), syngeneic species (isograft), the same species (allograft) or different species (xenograft). Such transplanted organs or tissues may be liver, kidney, heart, lung, combined heart-lung, trachea, spleen,

pancreatic (complete or partial, e.g. Langerhans islets), skin, small intestine, cornea, bone marrow, limb, muscle, nerve, intervertebral disc, myoblast or cartilage, or a combination of any of the foregoing.

The compound (I) or (II) for use in the preventing and/or treating of chronic rejection may be administered alone or in combination with one or more other immunosuppressive agents, for example cyclosporin A, tacrolimus, rapamycin, azathioprine, corticosteroids, anti-lymphocyte globulin or OKT3; especially cyclosporin A or tacrolimus, simultaneously, separately or sequentially. Further, the compound (I) or (II) for this use can be administered in a form of mixture in a pharmaceutical composition with one or more other immunosuppressive agents, mentioned above. Such combination or mixing remedy is included within the scope of this invention.

While the dosage of therapeutically effective amount of the compound (I) or (II) varies from and also depends upon the age and condition of each individual patient to be treated, a daily dose of about 1mg-10g/body, preferably 5mg-5g/body and more preferably 10mg-2g/body of the active ingredient is generally given for preventing and/or treating this disease, and an average single dose of about 0.5-1mg, 5mg, 10mg, 50mg, 100mg, 250mg, 500mg, 1g, 2g and 3g is generally administered. Daily dose for administration in humans for preventing or treating chronic rejection will be in the range of about 0.1-50mg/kg. In a combination or mixing remedy, for example, tacrolimus may be

administered in humans in a daily dose of about 0.01-5mg/kg, preferably 0.05-0.5mg/kg.

While the term for administering the compound (I) or (II) to prevent chronic rejection varies depending on species, and the nature and severity of the condition to be prevented, the compound (I) or (II) may usually be administered to humans for a short term or a long term, i.e. for 1 week to 1 year or more after transplantation, unless chronic rejection commences.

The possible mechanism of preventing and treating of chronic rejection in the compound (I) or (II) is associated with reduction of anti-glomeruli basement membrane (GBM) antibody, following by a sustained suppression of TGFβ.

The following examples illustrate the present invention in further detail. It should be understood that those examples are not intended to limit the scope of the invention.

Example 1. Prevention of chronic rejection

(1) METHOD

Inbred male Lewis rats (LEW) ($RT1^{I}$), weighing 250-300 g, were used as kidney transplantation recipients. Inbred male LEW and Fisher (F344) ($RT1^{IvI}$), weighing 250-350 g, were used as isograft and allograft donor rats, respectively. Kidney transplantation was performed using the modified technique of Fisher and Lee. [Fisher et al., Surgery, 58:904-914, 1965] Survival of kidney transplant was measured as time of recipient rat survival. Blood and 24 hr urine samples were collected once

a week for plasma creatinine, proteinuria, and the measurement of antibody titer against donor glomeruli basement membrane protein (GBM). Kidney grafts were harvested on the 90th day posttranspalantation and subjected to histology and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. The compound (I), at doses of 10 mg/kg and 20 mg/kg were administered orally to recipient rats daily from day 0 to day 9 after transplantation. Control isograft and allograft recipients received no drug after transplantation.

The recipient's kidney function was determined by measuring their plasma creatinine and proteinuria once a week for 90 days. Blood and urine samples were collected from recipients with kidney grafts described in the above. Plasma creatinine was tested by Sigma Creatinine Kit and proteinuria by Bio-Rad Protein assay.

Kidney graft tissues were harvested from recipients on day 90th after transplantation for histological analysis. Graft samples were fixed in 10% NBF and subsequently processed then immediately embedded in ParaPlast™ paraffin embedding media. Samples were sectioned at 3 μm, pre-warmed, deparaffinized, rehydrated, and subsequently stained in one of four processes: Hematoxylin and Eosin, Per-Iodic Acid Schiff, Verhoeff's Combined Elastic Trichrome, and Per-Iodic Acid Silver Methenamine. Histological sections were blindly evaluated by two histologists and scored semiquantitatively based on modified Banff' criteria for transplant pathology. [Solez et al., Kidney Int., 44:411-422, 1993]

TGFβ as been considered to play a crucial role for causing chronic allograft rejection. Kidney graft tissues harvested from recipients on day 90th after transplantation were subjected to RT-PCR for TGFβ gene expression. Total RNA was extracted from transplanted kidney tissues by TRIZOL. Real time RT-PCR was performed as described by Overbergh et al, [Overbergh et al., Cytokine, 11:305, 1999] using the ABI Prism 7700 sequence detection system and reagents from PE Biosystems, normalized to rodent GAPDH. The primers and probe for rat TGFβ were 5′-GCTGCTGACCCCCACTGAT-(sense), 5′-GCCACTGCCGGACAACTC-(anti sense), and CGCCTGAGTGGCTGTCTTTTGACGT-TAMRA. Rodent GAPDH primers and probe were designed by PE Biosystem.

Specific antibody against F344 rat glomeruli basement membrane protein in plasma from LEW recipients with F344 kidneys were also measured in the isograft, untreated allograft and allograft treated with the compound (I) at doses of 10 mg/kg and 20 mg/kg near days 20, 40, and 90 after transplantation by using ELISA assay.

(2) RESULT

. The isografts survived more than 90 days. In contrast, only 40% of the control allografts survived more than 90 days after grafting. The allografts of those receiving the compound (I) at dose of 10 mg/kg and the compound (I) at dose of 20 mg/kg survived more than 90 days post-transplantation were 80% and 100%, respectively. (Table 1)

Table 1.

Group	Drug	Route	Period	n	Survival day	Survival rate
Isograft	-	-	-	6	>90	100%
Allograft	-	-	-	10	14, 20, 21, 24, 38, 72, >90(4)	40%
Compound (I)	10mg/kg	PO	0-9 day	5	28, >90(4)	80%
Compound (I)	20mg/kg	PO	0-9 day	5	>90(5)	100%

In the absence of the compound (I) treatment, recipient plasma creatinine was increased by week 7 and proteinuria was positively detected by week 5. Both the compound (I) at doses of 10 mg/kg and 20 mg/kg treated recipients maintained normal creatinine and undetectable proteinuria as in the naïve rats and the isograft recipients during the period we followed. (Fig 1-4)

The untreated allograft control was observed for development of progressive histological chronic rejection. The approximate cumulative reduction in Banff' scores of kidney grafts from recipients treated with the compound (I) 10 mg/kg and 20 mg/kg are as following: interstitial inflammation 50% and 67%, tubulitis 100% and 100%, vasculitis 33% and 50%, mesangiolysis 83% and 100%, glomerulitis 75% and 38%, tubular atrophy 40% and 85%, glomerulosclerosis 83% and 100%, fibro-intimal hyperplasia 63% and 44%, and transplant glomerulopathy 79% and 100%, respectively, when compared with the untreated allograft control. And based on Banff' criteria of kidney transplant pathology, (-):Grade 0, Normal, (+):Grade 1, Mild, (++):Grade 2, Moderate

and (+++):Grade 3, Severe are used for diagnostic evaluation of chronic rejection. (Table 2)

Table 2.

Group	1*	2*	3*	4*	5*	6*	7*	8*	9*
Compound (I) 10 mg from day 0-9	+	-	+	-	+	+	-	++	-
Compound (I) 10 mg from day 0-9	++	-	+++	+	-	+	+	-	+
Compound (I) 20 mg from day 0-9	+	-	+++	-	++	+	-	++	-
Compound (I) 20 mg from day 0-9	+	-	+++	-	+	-	-	++	-
Compound (I) 20 mg from day 0-9	+	-	-	-	-	-	-	+	-
Compound (I) 20 mg from day 0-9	+	-	-	-	++	-	-	+	-
Allograft Control	+++	+	+++	+++	+	+	+++	++	++
Allograft Control	+++	++	+++	+++	+++	++	+++	+++	++
Allograft control	+++	++	+++	+++	++	++	+++	+++	+++

1*:Inflammation, 2*:Tubulitis, 3*:Vasculitis, 4*:Mesangiolysis, 5*:Glomerulitis, 6*:Tubular Atrophy, 7*:Glomerulosclerosis, 8*:Fibro-intimal Hyperplasia, 9*:Transplant Glomerulopathy

Compared with the isograft control, TGFβ mRNA was significantly up-regulated in the untreated allograft control. The compound (I) treatment inhibited TGFβ gene expression in a

dose-dependent manner on day 90 after grafting compared with the untreated allograft control. (Fig 5)

In the isograft control group, plasma anti-GBM was undetectable. It was detectable near day 20 after transplantation, increased thereafter in the untreated allograft control. Both the compound (I) at doses of 10 mg/kg and 20 mg/kg-treated recipients showed a trend of reduced production of antibody against donor GBM. (Fig 6-9)

Example 2. Prevention of chronic rejection in combination with tacrolimus

(1) METHOD

The rats and kidney transplantation methods described in Example 1 were used. The compound (I) at dose of 3 mg/kg and tacrolimus at dose of 1 mg/kg, were administered orally to recipient rats daily for 90 days after transplantation. The isograft, untreated allograft, and allograft treated with tacrolimus 1 mg/kg for 90 days alone served as control groups.

Blood and urine samples were collected once a week for 90 days from recipients with kidney grafts described in Example 1 for measuring their plasma creatinine and proteinuria. Plasma creatinine was tested by Sigma Creatinine Kit and proteinuria by Bio-Rad Protein assay.

Using the methods described in Example 1, histological changes of chronic allograft rejection were analyzed. Histological sections were blindly evaluated by two histologists

and scored semiquantitatively based on modified Banff' criteria for transplant pathology.

Specific antibody against F344 rat glomeruli basement membrane protein in plasma from LEW recipients with F344 kidneys were also measured in the isograft, untreated allograft and allograft treated with the compound (I) at dose of 3 mg/kg, in combination with tacrolimus at dose of 1mg/kg near day 20, 40, and 90 after transplantation by using methods described in Example 1.

(2) RESULT

The isografts survived more than 90 days. In contrast, only 40% of the control allografts survived 90 days after grafting. The allografts of those receiving tacrolimus at dose of 1 mg/kg and the compound (I) at dose of 3 mg/kg in combination with tacrolimus at dose of 1 mg/kg survived 90 days posttransplantation were both 100%. (Table 3)

Table 3.

Group	Drug	Route	Period	n	Survival day	Survival rate
Isograft	-	-	-	6	>90	100%
Allograft	-	-	-	10	14, 20, 21, 24, 38, 72, >90(4)	40%
Tacrolimus	1mg/kg	PO	0-90 day	4	>90	100%
Compound (I) Tacrolimus	3mg/kg 1mg/kg	PO PO	0-90 day	4	>90	100%

In the untreated allogenic transplantation, recipient plasma creatinine was increased by week 7 and proteinuria was positively detected by week 5. The compound (I) at dose of 3 mg/kg

in combination with tacrolimus at dose of 1 mg/kg-treated recipients showed decreased levels in both plasma creatinine and proteinuria compared with the untreated allograft control. (Fig 10, 11)

The untreated allograft control was observed for development of progressive histological chronic rejection. The approximate cumulative reduction in Banff' scores of kidney grafts from recipients treated with the compound (I) at dose of 3 mg/kg and tacrolimus at dose of 1 mg/kg are as following: interstitial inflammation 50%, tubulitis 85%, vasculitis 92%, mesangiolysis 75%, glomerulitis 38%, tubular atrophy 55%, glomerulosclerosis 58%, fibro-intimal hyperplasia 63%, and transplant glomerulopathy 57%, respectively, when compared with the untreated allograft control. And based on Banff' criteria of kidney transplant pathology, (-), (+), (++) and (+++) are defined same as Table 2. (Table 4)

Table 4.

Group	1*	2*	3*	4*	5*	6*	7*	8*	9*
Compound (I) 3 mg + Tacrolimus 1 mg for 90 days	+	-	+	-	+	+	-	++	-
Compound (I) 3 mg + Tacrolimus 1 mg for 90 days	++	-	+++	+	-	+	+	-	+
Compound (I) 3 mg + Tacrolimus 1 mg for 90 days	+	-	+++	-	++	+	-	++	-
Compound (I) 3 mg + Tacrolimus 1 mg for 90 days	+	-	+++	-	+	-	-	++	-
Allograft Control	+++	+	+++	+++	+	+	+++	++	++
Allograft Control	+++	++	+++	+++	+++	++	+++	+++	++
Allograft control	+++	++	+++	+++	++	++	+++	+++	+++

1*:Inflammation, 2*:Tubulitis, 3*:Vasculitis, 4*:Mesangiolysis, 5*:Glomerulitis, 6*:Tubular Atrophy, 7*:Glomerulosclerosis, 8*:Fibro-intimal Hyperplasia, 9*:Transplant Glomerulopathy

In the isograft control group, plasma anti-GBM was undetectable. It was detectable by day 20 after transplantation, increase thereafter in the untreated allograft control. The compound (I) at dose of 3 mg/kg, in combination with tacrolimus at dose of 1 mg/kg - treated recipients had no detectable levels of antibody against donor GBM, as in the isograft control group. (Fig 12)

Example 3. Treatment of chronic rejection

(1) METHOD

The rats and kidney transplantation methods described in Example 1 were used. The compound (I) at a dose of 20 mg/kg was administered orally to recipient rats for 3 weeks started from the time when they revealed either increased plasma creatinine or detectable proteinuria. The isograft and untreated allograft served as control groups. Blood and urine samples were collected once a week from recipients with kidney grafts described in Example 1 for measuring their plasma creatinine and proteinuria. Plasma creatinine was tested by Sigma Creatinine Kit and proteinuria by Bio-Rad Protein assay.

Using the methods described in Example 1, histological changes of chronic allograft rejection under rescue treatment of the compound (I) were analyzed. Histological sections were blindly evaluated by two histologists and scored semiquantitatively based on modified Banff' criteria for transplant pathology.

(2) RESULT

In the untreated allograft control, recipient plasma creatinine was increased by week 7 and proteinuria was positively detected by week 5. Although the compound (I) rescue treatment did not show an immediate improvement of recipient kidney function, both plasma creatinine and proteinuria tended to be at a normal level after drug treatment was discontinued. (Fig 13, 14)

The untreated allograft control was observed for

development of progressive histological chronic rejection. The approximate cumulative reduction in Banff' scores of kidney grafts from recipients treated with the compound (I) at dose of 20 mg/kg for 3 weeks during ongoing chronic allograft rejection are as following: interstitial inflammation 50%, tubulitis 70%, vasculitis 92%, mesangiolysis 33%, glomerulitis 38%, tubular atrophy 42%, fibro-intimal hyperplasia 53%, and transplant glomerulopathy 89%, respectively, when compared with the untreated allograft control. And based on Banff' criteria of kidney transplant pathology, (-), (+), (++) and (+++) are defined same as Table 2. (Table 5)

Table 5.

Group	1*	2*	3*	4*	5*	6*	7*	8*	9*
Compound (I) rescue From day 40-70	+	-	+	-	+	+	-	++	-
Compound (I) rescue From day 40-70	++	-	+++	+	-	+	+	-	+
Compound (I) rescue From day 40-70	+	-	+++	-	++	+	-	++	-
Compound (I) rescue From day 40-70	+	-	+++	-	+	-	-	++	-

1*:Inflammation, 2*:Tubulitis, 3*:Vasculitis, 4*:Mesangiolysis, 5*:Glomerulitis, 6*:Tubular Atrophy, 7*:Glomerulosclerosis, 8*:Fibro-intimal Hyperplasia, 9*:Transplant Glomerulopathy

Example 4. Treatment of chronic rejection in combination with brief treatment of tacrolimus

(1) METHOD

The rats and kidney transplantation methods described in Example 1 were used. Tacrolimus at dose of 1 mg/kg from day 0 to day 9 after transplantation, and the compound (I) at doses of 10 mg/kg and 15 mg/kg from day 28 to day 60 after transplantation were administered orally to recipient rats. In this study LEW recipients were briefly treated with oral tacrolimus at 1 mg/kg/day for 10 days after transplantation to avoid acute rejection and slow chronic rejection that gradually destroys the F344 kidney graft, resulting in functional and histological changes similar to the chronic rejection in human. The isograft, untreated allograft and allograft treated with tacrolimus 1 mg/kg for 10 days alone served as control groups. Blood and urine samples were collected once a week from recipients with kidney grafts described in Example 1 for measuring their plasma creatinine and proteinuria. Plasma creatinine was tested by Sigma Creatinine Kit and proteinuria by Bio-Rad Protein assay.

(2) RESULT

The isografts survived more than 90 days. In contrast, only 40% of the control allografts survived up to 90 days after grafting. The allografts of those receiving tacrolimus at dose of 1 mg/kg for 10 days alone after transplantation showed 100% of allograft survival rate. The individual allograft survival rates for recipients treated with a brief dose of tacrolimus and the compound (I) 10 mg/kg or 15 mg/kg from day 28 to day 60 after transplantation will be available after increasing of animal case number. (Table 6)

Table 6.

Group	Drug	Route	Period	n	Survival day	Survival rate
Isograft	-	-	-	6	>90	100%
Allograft	-	-	-	10	14, 20, 21, 24, 38, 72, >90(4)	40%
Tacrolimus	1mg/kg	PO	0-9 day	5	>90	100%
Tacrolimus Compound (I)	1mg/kg 10mg/kg	PO PO	0-9 day 28-60 day	2	>90(2)	N/A
Tacrolimus Compound (I)	1mg/kg 15mg/kg	PO PO	0-9 day 28-60 day	1	>90	N/A

The recipient's kidney function was determined by measuring their plasma creatinine and proteinuria once a week for 90 days. Plasma creatinine increased rapidly after week 7 post transplantation in the allograft control and week 8 in the allografts treated with a brief dose of tacrolimus, whereas, is remained within the normal range in the isograft control. The compound (I) 10 mg/kg from day 28 to day 60 maintained the plasma creatinine level less than the normal value of 1.5 mg/dL during the entire study period. Although the recipient treated with the compound (I) 15 mg/kg/day showed increased plasma creatinine started from week 3 to week 9 after transplantation, it was reversed and maintained in a normal level after that. (Fig 15, 16) Among the 40% of the allograft control rats and 100% of the allografts treated with a brief dose of tacrolimus survived more than 90 days after transplantation, preteinuria were detectable by week

2 and week 5, respectively after transplantation and dramatically increasing thereafter when compared with the isograft control. Both the compound (I) 10 mg/kg and 15 mg/kg treatment from day 28 to day 60 decreased the progression of proteinuria in kidney recipients. (Fig 17, 18)

The compound (I) or (II) was proved to have an activity to prevent and/or treat chronic rejection in a transplanted organ or tissue. So, the present invention provides useful immunosuppressant for preventing and/or treating chronic rejection in a transplanted organ or tissue.

Brief Description of Drawings

Fig 1 shows plasma creatinine concentrations after treatment with the compound (I) at dose of 10mg/kg. (Example 1)

Fig 2 shows plasma creatinine concentrations after treatment with the compound (I) at dose of 20mg/kg. (Example 1)

Fig 3 shows proteinuria quantities after treatment with the compound (I) at dose of 10mg/kg. (Example 1)

Fig 4 shows proteinuria quantities after treatment with the compound (I) at dose of 20mg/kg. (Example 1)

Fig 5 shows inhibition of TGFβ gene expression in treatment with the compound (I). (Example 1)

Fig 6 shows productions of antibody against GBM in syngeneic transplantation. (Example 1)

Fig 7 shows productions of antibody against GBM in allogeneic

transplantation. (Example 1)

Fig 8 shows productions of antibody against GBM in allogeneic transplantation treated with the compound (I) at dose of 10mg/kg. (Example 1)

Fig 9 shows productions of antibody against GBM in allogeneic transplantation treated with the compound (I) at dose of 20mg/kg. (Example 1)

Fig 10 shows plasma creatinine concentrations in transplantation treated with the compound (I) at dose of 3mg/kg in combination with tacrolimus at dose of 1mg/kg. (Example 2)

Fig 11 shows proteinuria quantities in transplantation treated with the compound (I) at dose of 3mg/kg in combination with tacrolimus at dose of 1mg/kg. (Example 2)

Fig 12 shows productions of antibody against GBM in allogeneic transplantation treated with the compound (I) in combination with tacrolimus. (Example 2)

Fig 13 shows plasma creatinine concentrations in transplantation treated with rescue the compound (I) at dose of 20mg/kg. (Example 3)

Fig 14 shows proteinuria quantities in transplantation treated with rescue the compound (I) at dose of 20mg/kg. (Example 3)

Fig 15 shows plasma creatinine concentrations in transplantation treated with the compound (I) at dose of 10mg/kg with brief treatment of tacrolimus. (Example 4)

Fig 16 shows plasma creatinine concentrations in

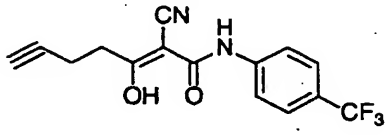
transplantation treated with the compound (I) at dose of 15mg/kg with brief treatment of tacrolimus. (Example 4)

Fig 17 shows proteinuria quantities in transplantation treated with the compound (I) at dose of 10mg/kg with brief treatment of tacrolimus. (Example 4)

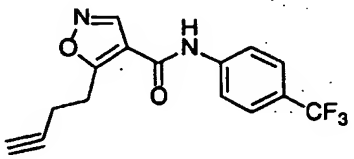
Fig 18 shows proteinuria quantities in transplantation treated with the compound (I) at dose of 15mg/kg with brief treatment of tacrolimus. (Example 4)

CLAIMS

1. A method for preventing and/or treating chronic rejection in a transplanted organ or tissue, which comprises administering a therapeutically effective amount of compound of the formula (I) or (II):



(I)



(II)

to a mammalian recipient in need thereof.

2. The method of claim 1 wherein the method is for preventing chronic rejection.

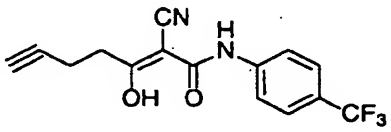
3. The method of claim 2 wherein the transplantation is allograft transplantation.

4. The method of claim 1 further comprising administering a therapeutically effective amount of tacrolimus.

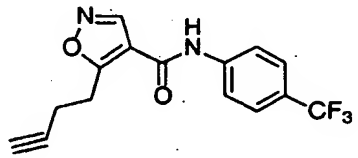
5. The method of claim 3 further comprising administering a therapeutically effective amount of tacrolimus.

6. The method of claim 1 wherein the method is in oral administration.

7. A use of a compound of the formula (I) or (II):



(I)



(II)

for the manufacture of a medicament for preventing and/or treating chronic rejection in a transplanted organ or tissue.

8. The use of claim 7 wherein the medicament is for preventing chronic rejection.

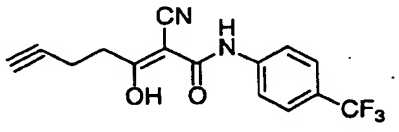
9. The use of claim 8 wherein the transplantation is allograft transplantation.

10. The use of claim 7 for the manufacture of the medicament with tacrolimus.

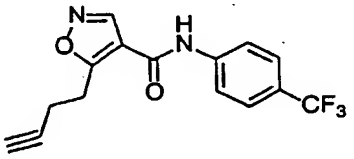
11. The use of claim 9 for the manufacture of the medicament with tacrolimus.

12. The use of claim 7 wherein the medicament is for oral administration.

13. A pharmaceutical composition for preventing and/or treating chronic rejection in a transplanted organ or tissue, which comprises a therapeutically effective amount of compound of the formula (I) or (II):



(I)



(II)

in admixture with a pharmaceutically acceptable carrier or excipient.

14. The pharmaceutical composition of claim 13 wherein the composition is for preventing chronic rejection.

15. The pharmaceutical composition of claim 14 wherein the transplantation is allograft transplantation.

16. The pharmaceutical composition of claim 13 which is for co-administering a therapeutically effective amount of tacrolimus.

17. The pharmaceutical composition of claim 15 which is for co-administering a therapeutically effective amount of tacrolimus.

18. The pharmaceutical composition of claim 13 which is for oral administration.

Fig 1

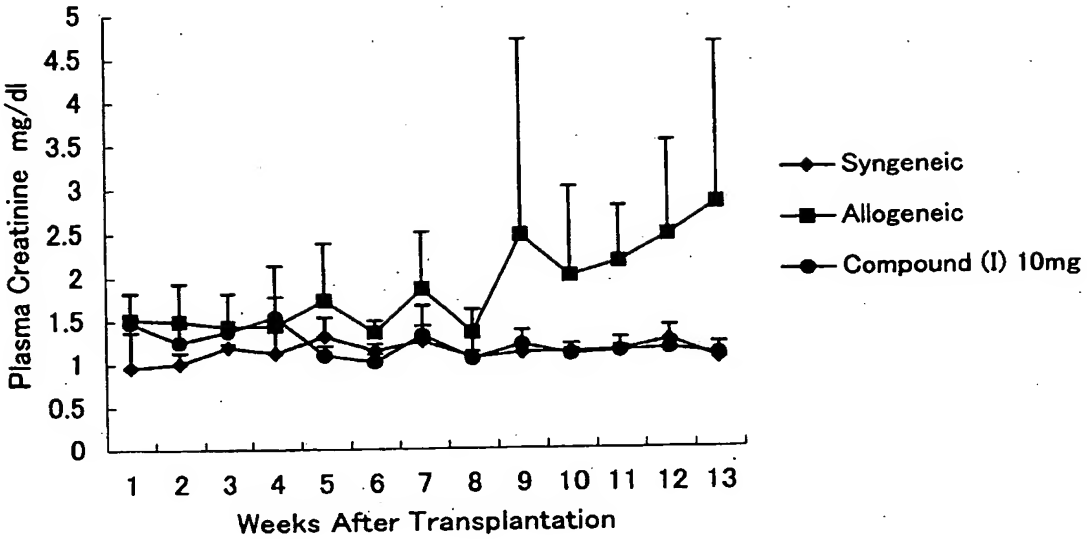

Plasma Creatinine mg/dl
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13
Weeks After Transplantation
Syngeneic
Allogeneic
Compound (I) 10mg

Fig 2

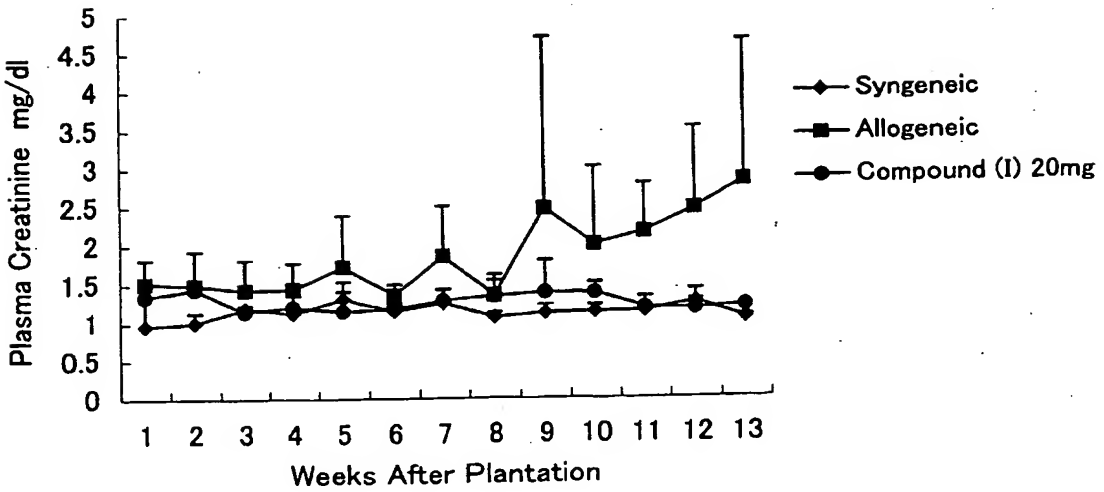

Plasma Creatinine mg/dl
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13
Weeks After Plantation
Syngeneic
Allogeneic
Compound (I) 20mg

Fig 3

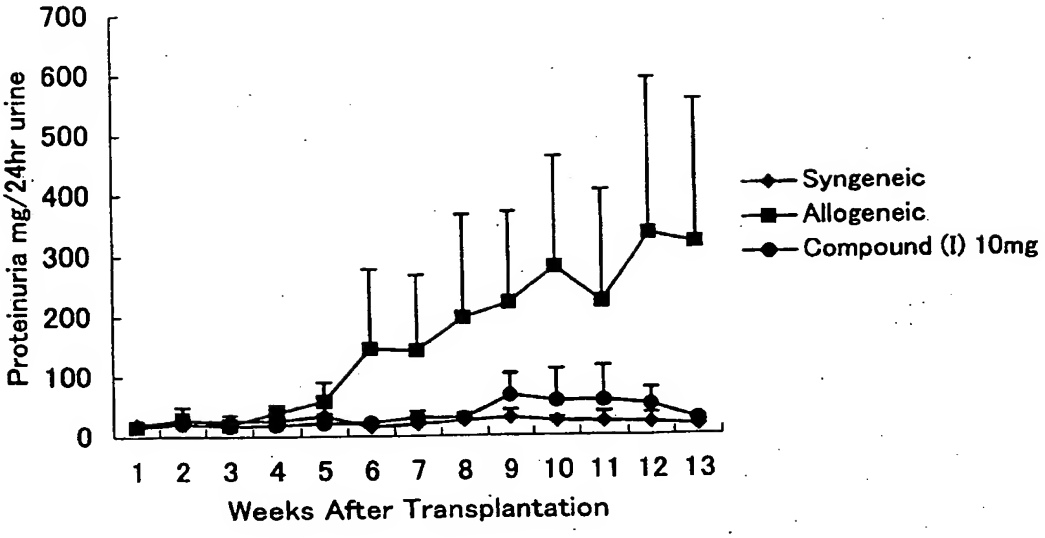

700
600
500
400
300
200
100
0
Proteinuria mg/24hr urine
1 2 3 4 5 6 7 8 9 10 11 12 13
Weeks After Transplantation
Syngeneic
Allogeneic
Compound (I) 10mg

Fig 4

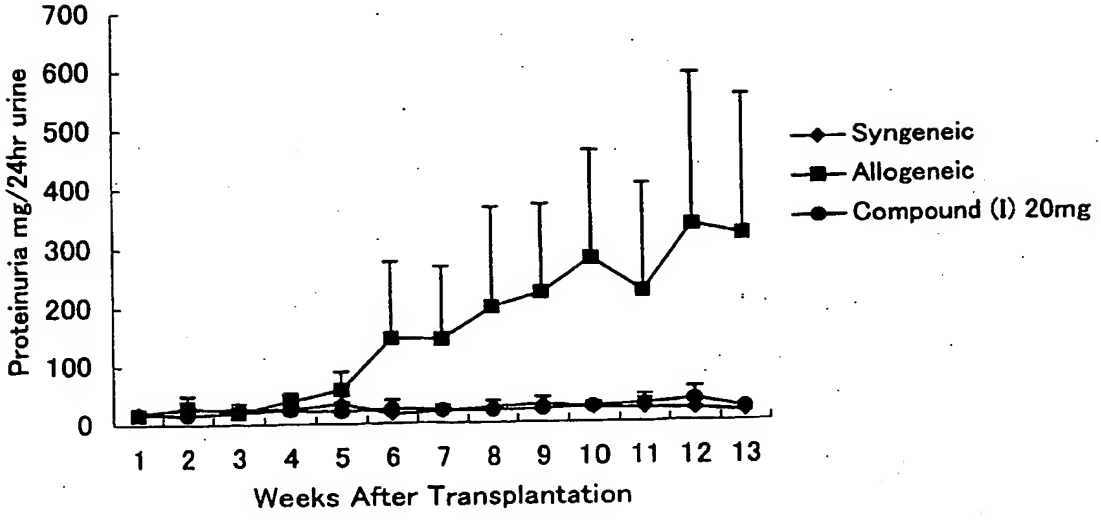

700
600
500
400
300
200
100
0
Proteinuria mg/24hr urine
1 2 3 4 5 6 7 8 9 10 11 12 13
Weeks After Transplantation
Syngeneic
Allogeneic
Compound (I) 20mg

Fig 5

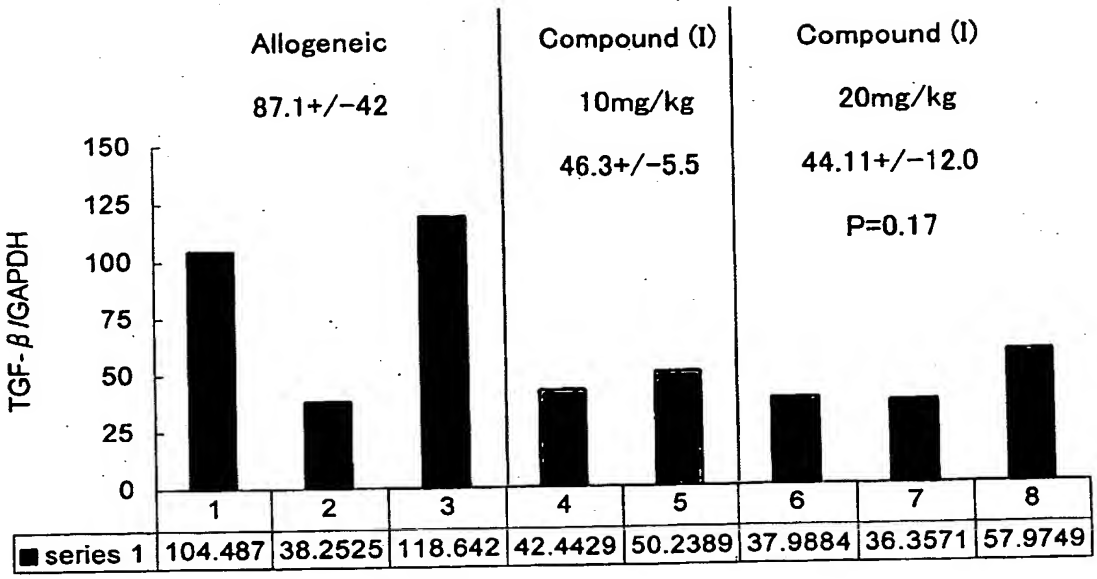

Allogeneic
87.1+/−42
Compound (I)
10mg/kg
46.3+/−5.5
Compound (I)
20mg/kg
44.11+/−12.0
P=0.17
TGF-β/GAPDH
150
125
100
75
50
25
0
1 2 3 4 5 6 7 8
series 1 104.487 38.2525 118.642 42.4429 50.2389 37.9884 36.3571 57.9749

Fig 6

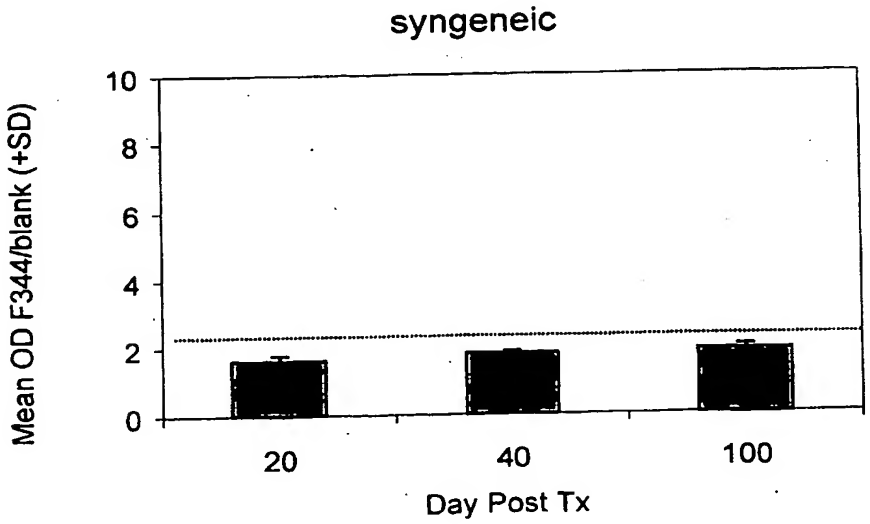

syngeneic
Mean OD F344/blank (+SD)
10
8
6
4
2
0
20
40
100
Day Post Tx

Fig 7

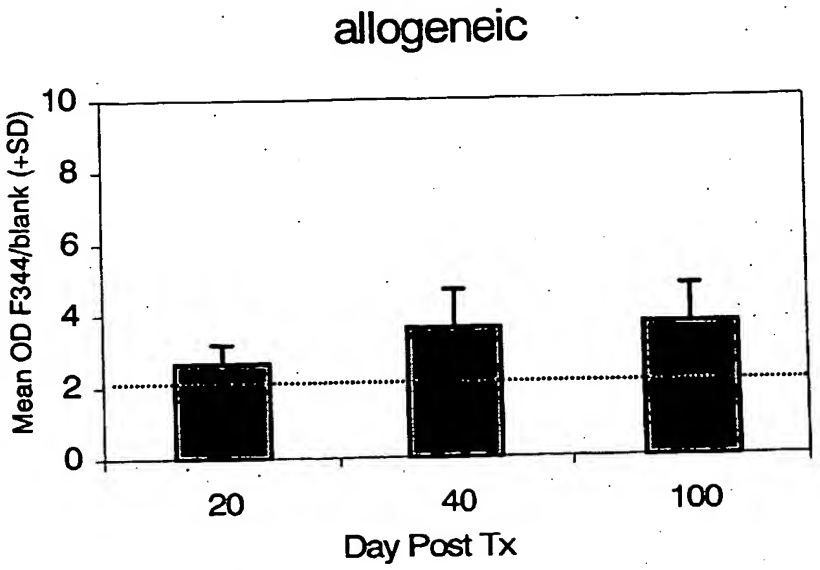

allogeneic
Mean OD F344/blank (+SD)
10
8
6
4
2
0
20
40
100
Day Post Tx

Fig 8

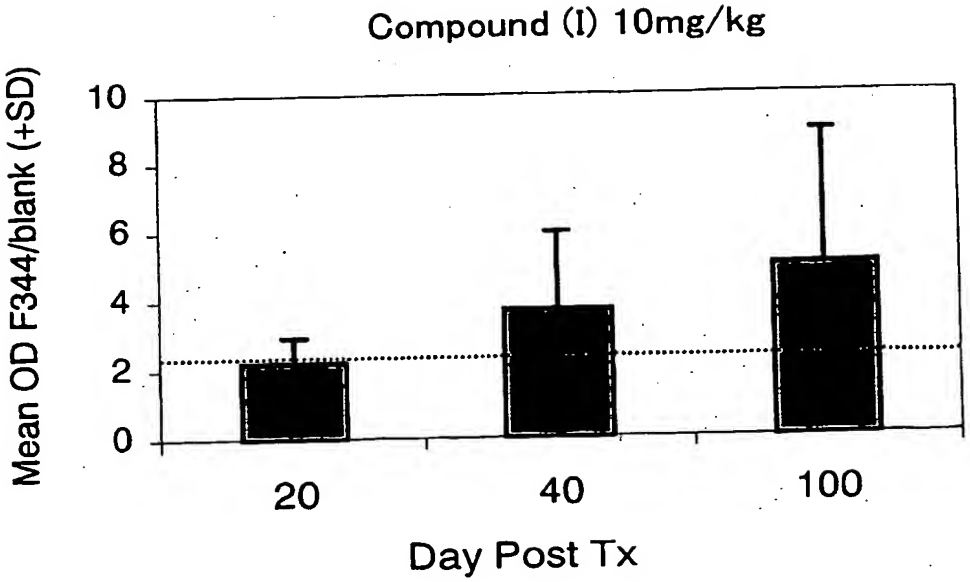

Compound (I) 10mg/kg
Mean OD F344/blank (+SD)
10
8
6
4
2
0
20
40
100
Day Post Tx

Fig 9

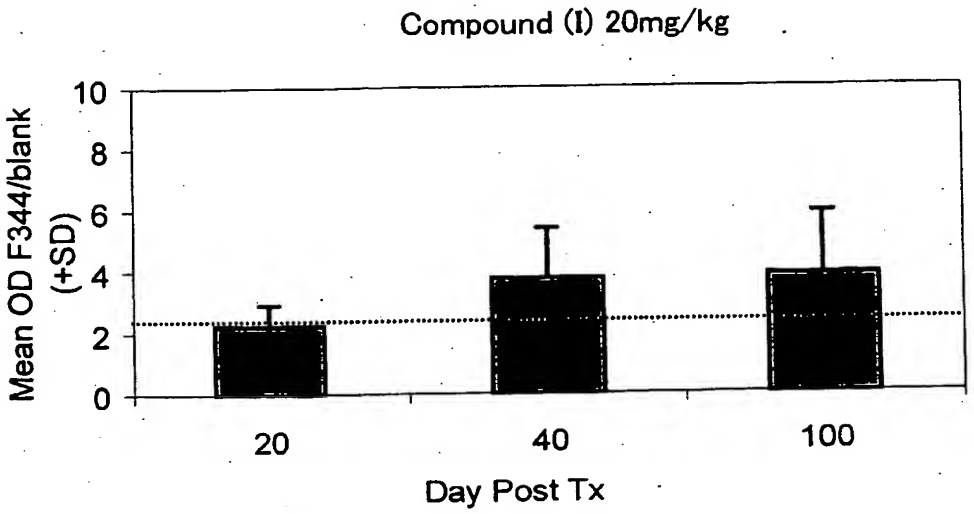

Compound (I) 20mg/kg
Mean OD F344/blank (+SD)
10
8
6
4
2
0
20
40
100
Day Post Tx

Fig 10

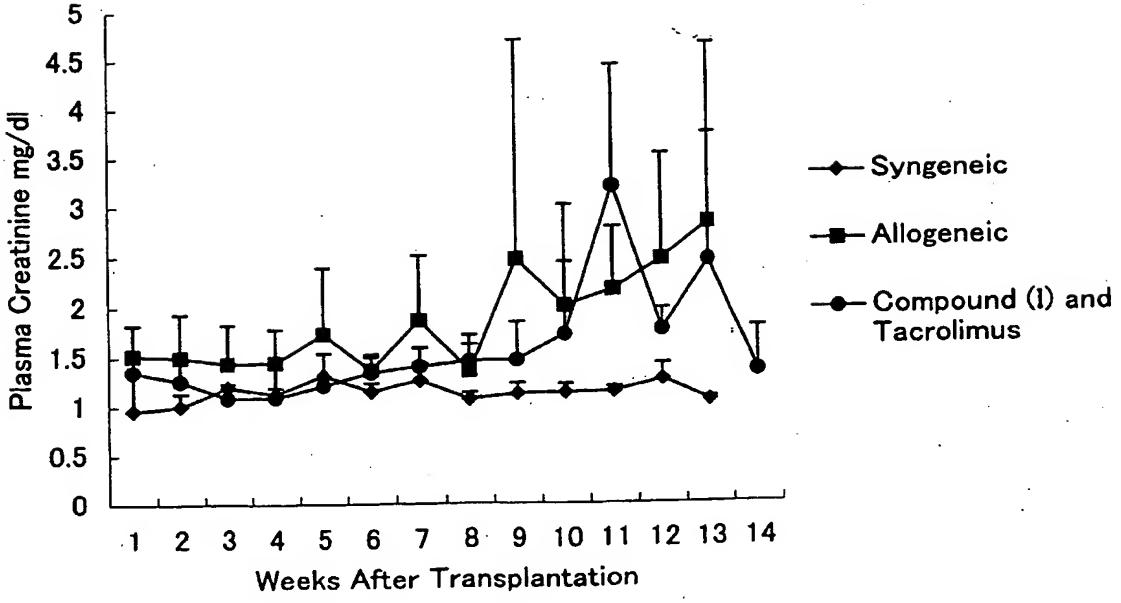

Plasma Creatinine mg/dl
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Weeks After Transplantation
Syngeneic
Allogeneic
Compound (I) and Tacrolimus

Fig 11

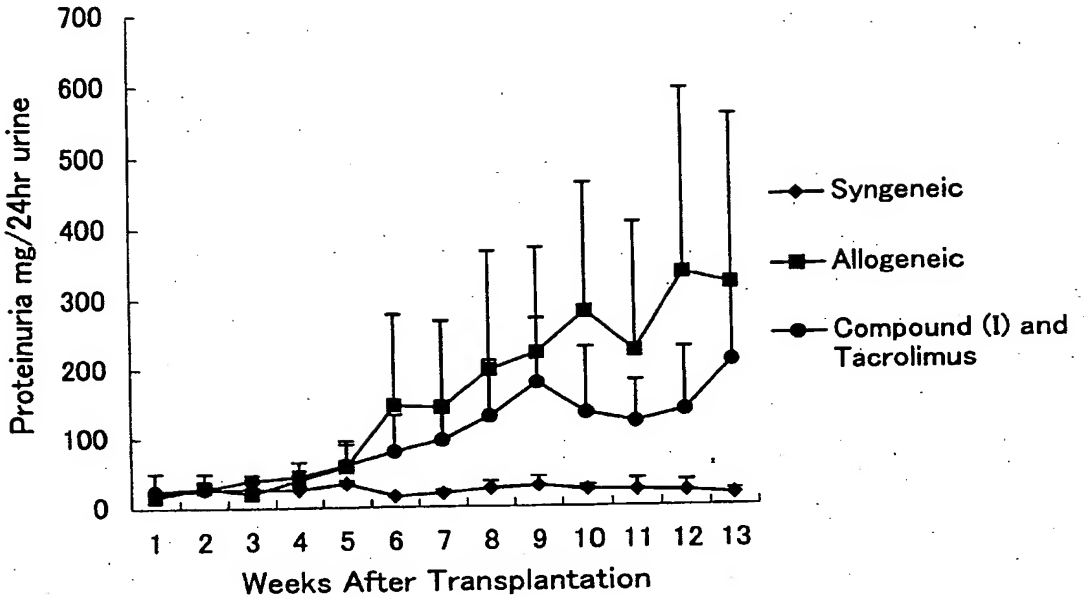

Proteinuria mg/24hr urine
700
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13
Weeks After Transplantation
Syngeneic
Allogeneic
Compound (I) and Tacrolimus

Fig 12

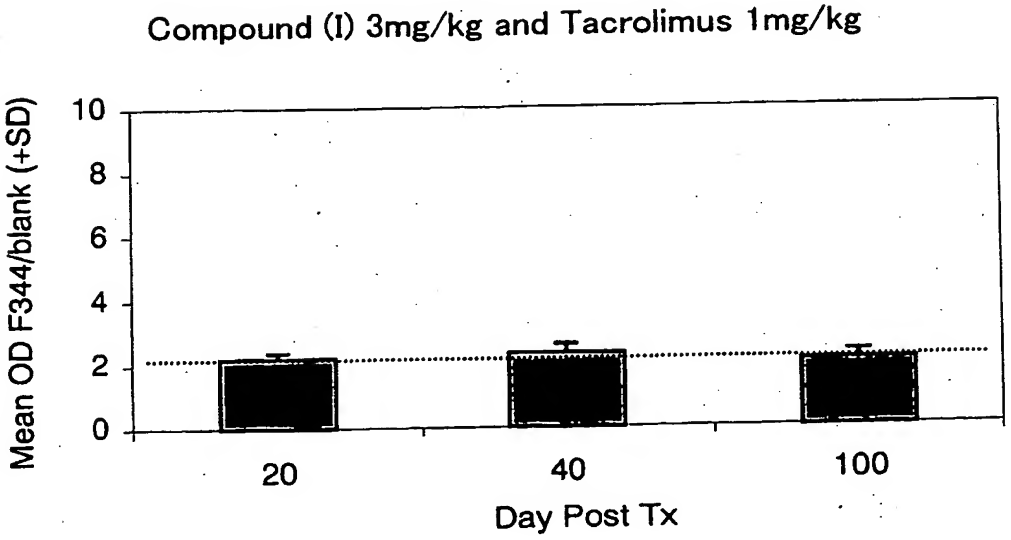

Compound (I) 3mg/kg and Tacrolimus 1mg/kg
Mean OD F344/blank (+SD)
10
8
6
4
2
0
20
40
100
Day Post Tx

Fig 13

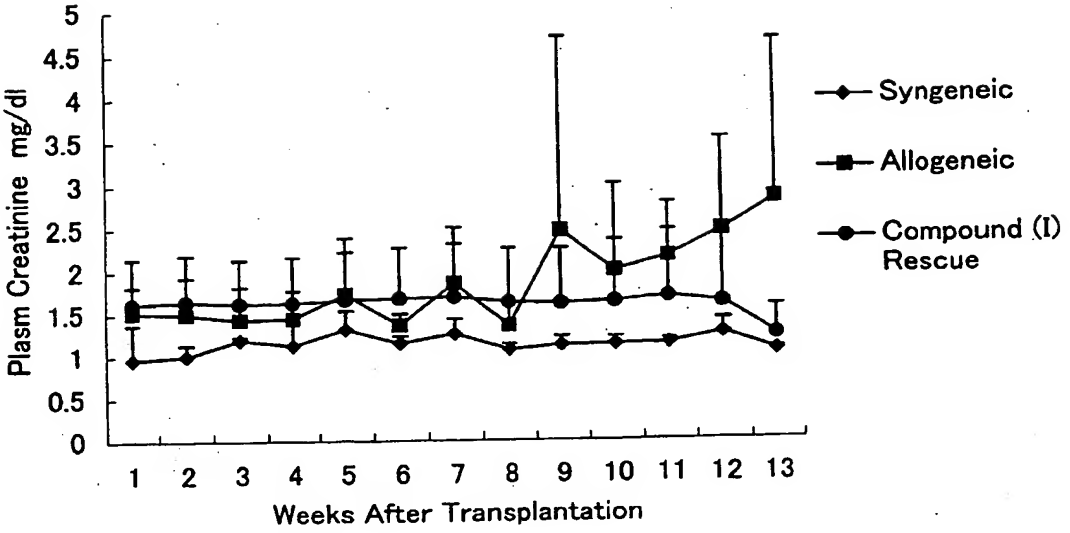

Plasm Creatinine mg/dl
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 13
Weeks After Transplantation
Syngeneic
Allogeneic
Compound (I) Rescue

Fig 14

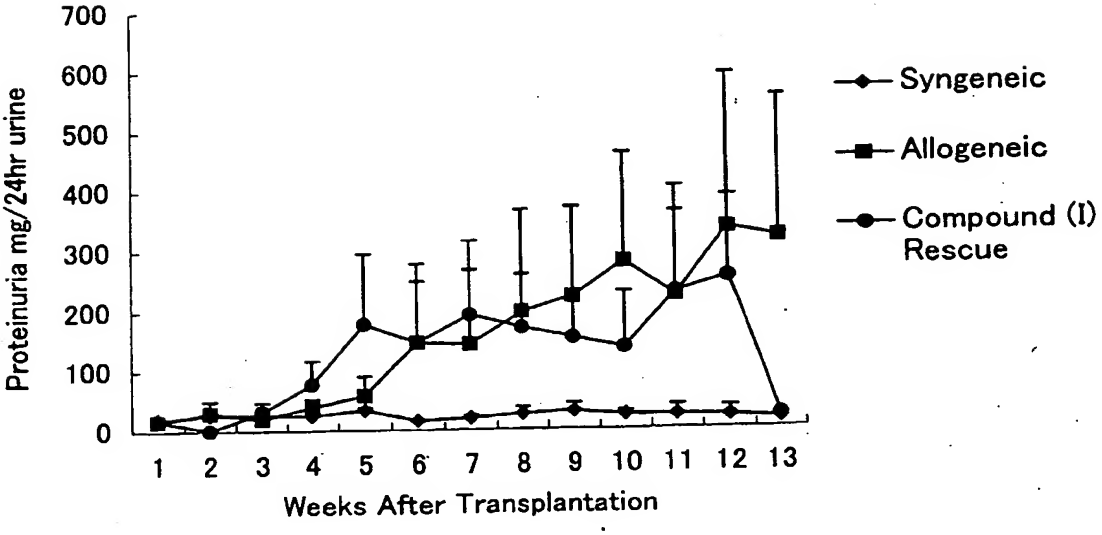

Proteinuria mg/24hr urine
700
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13
Weeks After Transplantation
Syngeneic
Allogeneic
Compound (I) Rescue

Fig 15

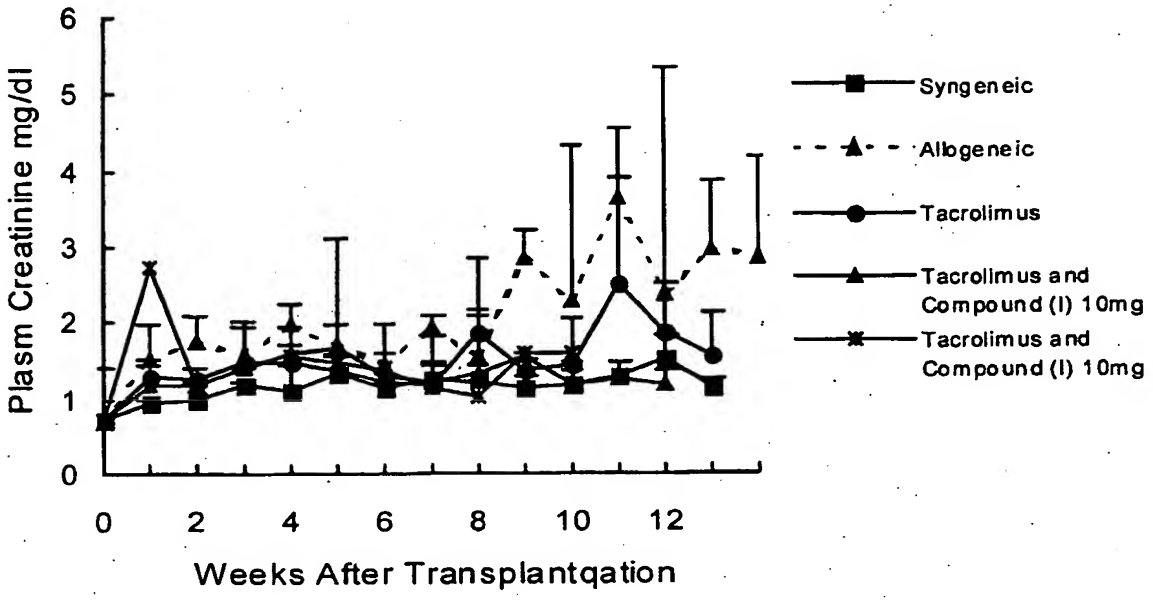

Plasm Creatinine mg/dl
0
1
2
3
4
5
6
0 2 4 6 8 10 12
Weeks After Transplantqation
Syngeneic
Allogeneic
Tacrolimus
Tacrolimus and Compound (I) 10mg
Tacrolimus and Compound (I) 10mg

Fig 16

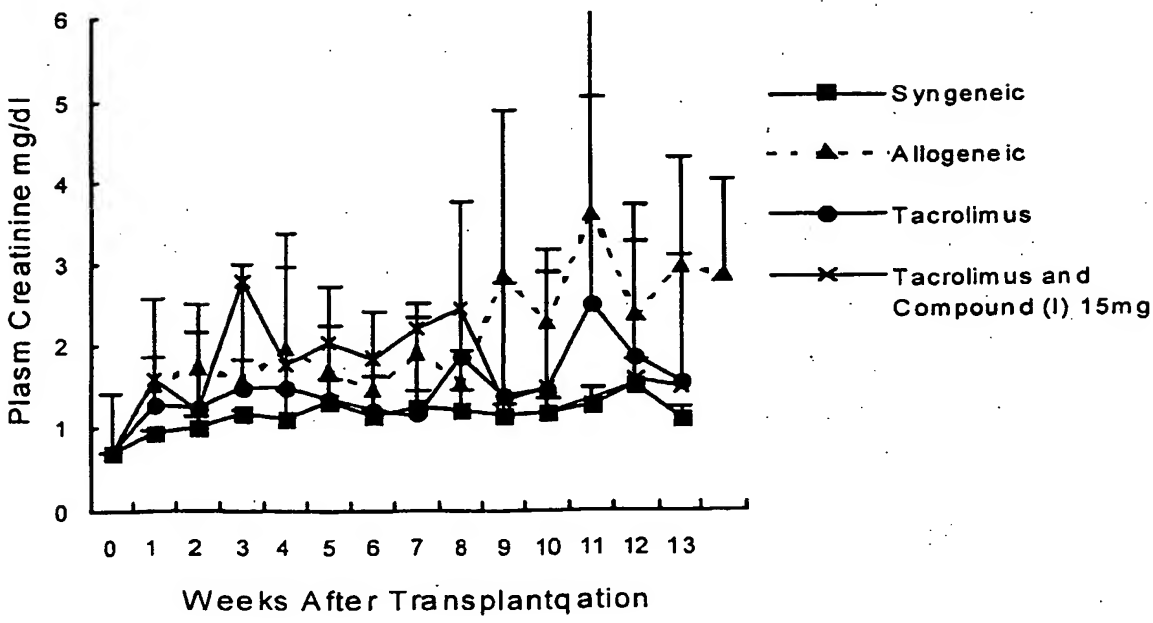

Plasm Creatinine mg/dl
0
1
2
3
4
5
6
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Weeks After Transplantqation
Syngeneic
Allogeneic
Tacrolimus
Tacrolimus and Compound (I) 15mg

Fig 17

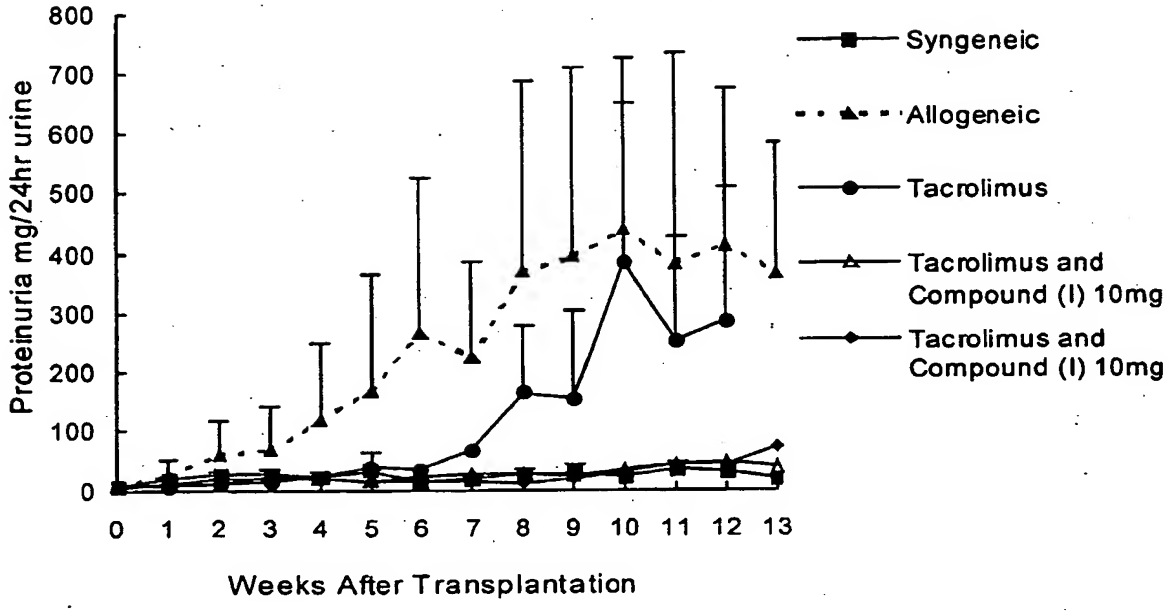
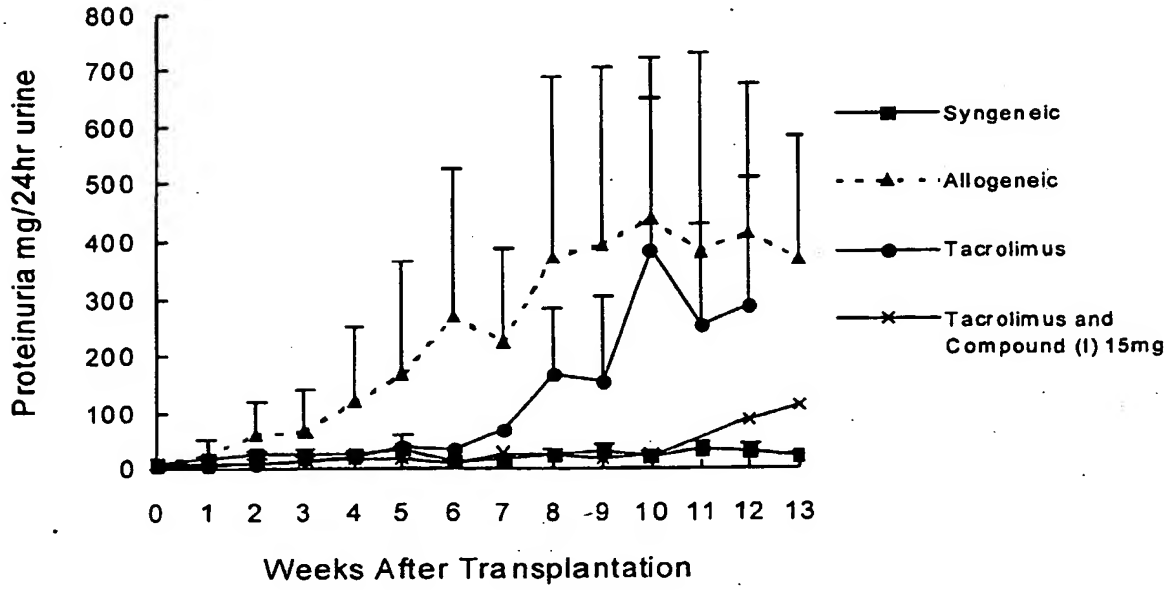

Proteinuria mg/24hr urine
800
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Weeks After Transplantation
Syngeneic
Allogeneic
Tacrolimus
Tacrolimus and Compound (I) 10mg
Tacrolimus and Compound (I) 10mg

Fig 18


Proteinuria mg/24hr urine
800
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Weeks After Transplantation
Syngeneic
Allogeneic
Tacrolimus
Tacrolimus and Compound (I) 15mg

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 03/04722

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/42 A61K31/275 A61P37/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 780 128 A (HOECHST AG) 25 June 1997 (1997-06-25) claims 1-10; example 3 ---	1-18
X	QI, Z. AND ALL.: "Malononitrilamides 715 and 279 prolong rat cardiac allograft survival, reverse ongoing rejection, inhibit allospecific antibody production and interact positively with cyclosporin" SKAN. J. IMMUNOL., vol. 48, 1998, pages 379-388, XP002247747 the whole document --- -/--	1-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 July 2003	29/07/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stienon, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 03/04722

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	QI, Z. AND ALL.: "Malonitrilamides 715 and 279 prevent accelerated cardiac allograft rejection synergistically with cyclosporin A in presensitized rats" TRANSPLANT IMMUNOLOGY, vol. 6, 1998, pages 94-100, XP002247748 the whole document	1-18
X	QI, Z. AND ALL.: "Malononitrilamides and tacrolimus additively prevent acute rejection in rat cardiac allografts" TRANSPLANT IMMUNOLOGY, vol. 7, 1999, pages 169-175, XP002247776 the whole document	1-18
A	SCHORLEMMER: "Malononitrilamides: a New strategy of Immunosuppression for Allo- and Xenotransplantation" TRANSPLANTATION PROCEEDINGS, vol. 30, 1998, pages 884-890, XP002247749 us the whole document	
A	WAAGA A M ET AL: "Mechanisms of chronic rejection" CURRENT OPINION IN IMMUNOLOGY, CURRENT BIOLOGY LTD, XX, vol. 12, no. 5, 1 October 2000 (2000-10-01), pages 517-521, XP004257715 ISSN: 0952-7915	
A	US 5 688 824 A (WILLIAMS JAMES) 18 November 1997 (1997-11-18) cited in the application	
A	US 5 624 946 A (WILLIAMS JAMES) 29 April 1997 (1997-04-29) cited in the application	
A	EP 0 665 013 A (HOECHST AG) 2 August 1995 (1995-08-02) cited in the application	
A	US 5 308 865 A (BARTLETT ROBERT R ET AL) 3 May 1994 (1994-05-03) cited in the application	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP 03/04722

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-6 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/JP 03/04722

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0780128 A	25-06-1997	DE 19547648 A1	26-06-1997
		EP 0780128 A2	25-06-1997
		JP 9188658 A	22-07-1997
		US 5780592 A	14-07-1998
US 5688824 A	18-11-1997	US 5624946 A	29-04-1997
		AU 2954195 A	25-01-1996
		WO 9601111 A1	18-01-1996
US 5624946 A	29-04-1997	AU 2954195 A	25-01-1996
		WO 9601111 A1	18-01-1996
		US 5688824 A	18-11-1997
EP 0665013 A	02-08-1995	US 5519042 A	21-05-1996
		AT 207355 T	15-11-2001
		AU 696851 B2	17-09-1998
		AU 1015095 A	20-07-1995
		CA 2140106 A1	14-07-1995
		DE 69523350 D1	29-11-2001
		DE 69523350 T2	04-07-2002
		DK 665013 T3	18-02-2002
		EP 0665013 A1	02-08-1995
		ES 2166381 T3	16-04-2002
		JP 2843774 B2	06-01-1999
		JP 7277976 A	24-10-1995
		PT 665013 T	28-03-2002
US 5308865 A	03-05-1994	AT 125252 T	15-08-1995
		AU 652088 B2	11-08-1994
		AU 3103593 A	15-07-1993
		BR 9300035 A	13-07-1993
		CA 2086908 A1	09-07-1993
		CN 1079216 A ,B	08-12-1993
		CZ 9203826 A3	16-03-1994
		CZ 285586 B6	15-09-1999
		DE 69300261 D1	24-08-1995
		DK 551230 T3	10-03-1997
		EP 0551230 A1	14-07-1993
		ES 2074916 T3	16-09-1995
		FI 930048 A	09-07-1993
		HU 63377 A2	30-08-1993
		IL 104225 A	04-01-1998
		JP 3145824 B2	12-03-2001
		JP 5310672 A	22-11-1993
		KR 276182 B1	15-12-2000
		NO 930036 A ,B,	09-07-1993
		NZ 245631 A	22-12-1994
		RU 2112772 C1	10-06-1998
		ZA 9300134 A	10-01-1994